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CLAIMS

- 1/ The use of a membrane fraction of Gram-negative bacteria for preparing a pharmaceutical composition which is immunostimulant and/or capable of inducing an antitumor immune response.
- 2/ The use as claimed in claim 1, characterized in that the membrane fraction comprises a membrane fraction of *Klebsiella pneumoniae*.
- 3/ The use as claimed in claim 1 or 2, characterized in that the membrane fraction comprises at least membrane fractions of two different strains of bacteria.
- 4/ The use as claimed in one of claims 1 to 3, characterized in that the membrane fraction is prepared using a method comprising the following steps:
- a) culturing of said bacteria in a culture medium which allows their growth, followed by centrifugation of said culture;
  - b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), then centrifugation of the suspension obtained;
  - c) extraction and elimination of the non-membrane-bound proteins and of the nucleic acids of the pellet obtained in step a) or b) with at least one cycle of washing the pellet in an extraction solution;
  - d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;
  - e) at least one cycle of washing the pellet obtained in step d) in a physiological solution and/or in distilled water; and

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f) ultrasonication of the pellet obtained in step e).

5/ The use as claimed in one of claims 1 to 3,  
5 characterized in that the membrane fraction is prepared using a method comprising the following steps:

10 a) culturing of said bacteria in a culture medium which allows their growth, followed, where appropriate, by centrifugation;

b) freezing of the culture medium or of the pellet obtained in step a), followed by thawing and drying of the cells;

15 c) elimination, using a DNase, of the nucleic acids from the dried cells obtained in step b), which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

20 e) precipitation, in acid medium, of the suspension obtained in step d) and elimination of the pellet;

25 f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

30 6/ The use as claimed in claim 2, characterized in that the membrane fraction is the *Klebsiella pneumoniae* P40 protein of sequence SEQ ID No. 2, or a fragment thereof.

35 7/ The use as claimed in one of claims 1 to 6, characterized in that the pharmaceutical composition also comprises an agent for vehiculing said membrane fraction in a form which makes it possible to improve its stability and/or its

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immunostimulant activity and/or its capacity to induce an antitumor immune response.

- 5        8/    The use as claimed in claim 7, characterized in that said agent is of the oil-in-water or water-in-oil emulsion type.
- 10       9/    The use as claimed in claim 7, characterized in that said agent is in the form of a particle of the liposome, microsphere or nanosphere type, or any type of structure which enables said membrane fraction to be encapsulated and presented in particulate form.
- 15       10/   The use as claimed in one of claims 1 to 9, characterized in that the pharmaceutical composition also comprises an agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions.
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- 25       11/   The use as claimed in claim 11 [sic], characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a cytokine.
- 30       12/   The use as claimed in claim 10, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a regulatory agent chosen from hormones.
- 35       13/   The use as claimed in claim 10, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a regulatory agent chosen from growth factors.

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- 14/ The use as claimed in claim 10, characterized in that the agent for potentiating the immunostimulant activity and/or the antitumor immune response of said membrane fractions is a cellular compound.
- 15/ The use as claimed in claim 14, characterized in that said cellular compound is a nucleic acid chosen from DNAs and RNAs.
- 16/ The use as claimed in claim 14, characterized in that said cellular compound is a compound of the ribosome family.
- 17/ The use as claimed in claim 14, characterized in that said cellular compound is a protein of the heat-shock protein family.
- 18/ The use as claimed in one of claims 1 to 17, for preparing a pharmaceutical composition intended to be administered in combination with an anticancer treatment.
- 19/ The use as claimed in claim 18, characterized in that the anticancer treatment is chemotherapy and/or radiotherapy.
- 20/ The use as claimed in either of claims 18 and 19, for preparing a pharmaceutical composition intended to be administered simultaneously with, separately from or spread out over time with the anticancer treatment.
- 21/ The use as claimed in claim 20, characterized in that the pharmaceutical composition is administered via the enteral or parenteral route.
- 22/ The use as claimed in one of claims 18 to 21, characterized in that said combined anticancer treatment is a chemotherapeutic treatment

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comprising a protease inhibitor or a compound with anti-angiogenic activity.

5 23/ The use as claimed in one of claims 1 to 22, for preventing and/or treating cancers.

10 24/ The use as claimed in claim 23, for preventing and/or treating bladder cancers, prostate cancers, colon cancers, liver cancers and malignant melanomas.

15 25/ A method for preparing a membrane fraction of Gram-negative bacteria, characterized in that it comprises the following steps:

- 20 a) culturing of said bacteria in a culture medium which allows their growth, followed by centrifugation of said culture;
- 25 b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), then centrifugation of the suspension obtained;
- 30 c) extraction and elimination of the non-membrane-bound proteins and of the nucleic acids of the pellet obtained in step a) or b) with at least one cycle of washing the pellet in an extraction solution;
- 35 d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;
- e) at least one cycle of washing the pellet obtained in step d) in a physiological solution and/or in distilled water; and
- f) ultrasonication of the pellet obtained in step e).

26/ A method for preparing a membrane fraction of Gram-negative bacteria, characterized in that it comprises the following steps:

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- a) culturing of said bacteria in a culture medium which allows their growth, followed, where appropriate, by centrifugation;
- 5 b) freezing of the culture medium or of the pellet obtained in step a), followed by thawing and drying of the cells;
- c) elimination, using a DNase, of the nucleic acids from the dried cells obtained in step b),
- 10 which have been resuspended;
- d) grinding of the cells obtained in step c) and clarification of the suspension obtained;
- e) precipitation, in acid medium, of the suspension obtained in step d) and elimination
- 15 of the pellet;
- f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and
- 20 g) sterilization of the concentrated membrane suspension obtained in step f).

27/ The method as claimed in claim 25 or 26, characterized in that said Gram-negative bacterium

25 is *Klebsiella pneumoniae*.

28/ A membrane fraction which can be obtained using a method as claimed in one of claims 25 to 27.

30 29/ A pharmaceutical composition comprising a membrane fraction as claimed in claim 28.

30/ A pharmaceutical composition comprising a membrane fraction of a Gram-negative bacterium, in

35 particular of *Klebsiella pneumoniae*, or a pharmaceutical composition as claimed in claim 29, characterized in that it is combined with an anticancer treatment by chemotherapy and/or by radiotherapy.

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31/ The pharmaceutical composition as claimed in claim  
30, characterized in that it contains an  
anticancer compound as a combination product for  
5 use which is simultaneous, separate or spread out  
over time.

32/ The pharmaceutical composition as claimed in claim  
30, characterized in that said anticancer compound  
10 is chosen from protease inhibitors or from  
compounds with anti-angiogenic activity.

ORIGINAL [signature]

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